DATA EVALUATION RECORD
WHOLE SEDIMENT ACUTE TOXICITY INVERTEBRATES, FRESHWATER
OPPTS Guideline 850.1735

1. **CHEMICAL:** Cypermethrin PC Code No.: 109702

2. **TEST MATERIAL:** Cypermethrin Technical 40/60 Purity: 40.6% cis/59.4% trans

3. **CITATION:**

Authors:	Picard, C.R.
Title:	10-Day Toxicity Test Exposing Freshwater Amphipods (*Hyalella azteca*) to Cypermethrin Applied to Glen Charlie Pond Sediment Under Static-Renewal Conditions.
Study Completion Date:	May 7, 2009
Laboratory:	Springborn Smithers Laboratories 790 Main Street Wareham, MA 02571
Sponsor:	Pyrethroid Working Group Beverage & Diamond 1350 I Street NW Washington, DC 20005
Laboratory Report ID:	13656.6130
MRID No.:	47946601
DP Barcode:	420006

4. **REVIEWED BY:** Christie E. Padova, Staff Scientist, Dynamac Corporation

Signature: Christie E. Padova **Date:** 06/07/10

APPROVED BY: Teri S. Myers, Senior Scientist, Cambridge Environmental Inc.

Signature: Teri S Myers **Date:** 06/09/10

5. **APPROVED BY:** Stephen Carey, Biologist, EPA/OCSPP/OPP/EFED/ERB6

Signature: Stephen Carey **Date:** 7-21-15

6. **STUDY PARAMETERS:**

Age of Test Organism:	7 to 8 days old
Definitive Test Duration:	10 days
Study Method:	Intermittent flow-through
Type of Concentrations:	Mean-measured sediment, bulk, and OC-normalized

7. CONCLUSIONS:

Results Synopsis:

Based upon mean-measured sediment concentrations:

Survival:
LC_{50}: 54 µg a.i./kg — 95% C.I.: 49 to 59 µg a.i./kg
Slope: N/A
NOAEC: 25 µg a.i./kg
LOAEC: 44 µg a.i./kg

Growth (dry weight):
EC_{50}: 31 µg a.i./kg — 95% C.I.: 26 to 37 µg a.i./kg
Slope: 4.99±1.25
NOAEC: 4.7 µg a.i./kg
LOAEC: 8.7 µg a.i./kg

Based upon ESTIMATED[1] pore water concentrations:

Survival:
LC_{50}: 0.007 µg a.i./L — 95% C.I.: 0.007 to 0.008 µg a.i./L
Slope: N/A
NOAEC: 0.003 µg a.i./L
LOAEC: 0.006 µg a.i./L

Growth (dry weight):
IC_{50}: 0.004 µg a.i./L — 95% C.I.: 0.004 to 0.005 µg a.i./L
Slope: 4.99±1.25
NOAEC: 0.0006 µg a.i./L
LOAEC: 0.001 µg a.i./L

Based upon OC-normalized mean-measured sediment concentrations:

Survival:
LC_{50}: 1060 µg a.i./kg TOC — 95% C.I.: 960 to 1160 µg a.i./kg TOC
Slope: N/A
NOAEC: 490 µg a.i./kg TOC
LOAEC: 860 µg a.i./kg TOC

1 Freely dissolved pore water endpoints (ug/L) estimated as:
Mean measured bulk sediment conc. (*ug/kg-dw*) / [Fraction TOC (*kg OC/kg-dw*) * K_{OC} (*L/kg-OC*)]

Growth (dry weight):
EC_{50}: 608 µg a.i./kg TOC 95% C.I.: 510 to 725 µg a.i./kg TOC
Slope: 4.99±1.25
NOAEC: 92 µg a.i./kg TOC
LOAEC: 170 µg a.i./kg TOC

8. ADEQUACY OF THE STUDY:

A. Classification: Acceptable
B. Rationale: N/A
C. Repairability: N/A

9. MAJOR GUIDELINE DEVIATIONS:

No major deviations noted.

10. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: *H. azteca* or *Chironomus tentans*	*Hyalella azteca*
Life Stage: For *C. tentans*: third instar (9-11 days old). The instar stage of midges must be confirmed by head capsule width (approx. 0.38 mm). For *H. azteca*: 7- to 14-day old amphipods must be produced. If growth is also an endpoint, a narrower range, such as 1- to 2-day old amphipods should be used.	7 to 8 days old
Supplier Brood stock can be obtained from laboratory, commercial, or government sources. (Sources obtained from the wild should be avoided unless cultured through several generations in the laboratory.)	Amphipods originated from laboratory cultures maintained in *ca.* 15 L of culture water (same source as dilution water) under flow-through conditions.
All organisms from the same source?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period: The required culture and testing temperature is 23°C. The test organisms should be cultured in the same water to be used for testing.	Adults were removed from the main culture tanks 8 to 9 days prior to test initiation and placed in *ca.* 8 L of water. Juvenile amphipods (<24 hours old) produced by the isolated adults were then transferred to *ca.* 0.80 L of laboratory dilution water and reared under static conditions for 7 to 8 days with gentle aeration. During the holding period, the dissolved oxygen ranged from 7.9 to 8.6 mg/L and temperature ranged from 22 to 24 °C.
Feeding:	During holding and acclimation, amphipods were fed every other day with 2.5 mL of a combination of yeast, cereal leaves, and flaked fish food suspension (YCT) and 2.5 mL of *Ankistrodesmus falcatus*.
Pretest Mortality: A group of organisms should not be used if they appear unhealthy, discolored (eg <20% mortality 48 h before the beginning of a test).	No mortality during the 48 hours prior to test initiation.

C. Test System

Guideline Criteria	Reported Information
Source of dilution water (overlying water) and sediment: Soft reconstituted water or water from a natural source. Tap water is acceptable if it is dechlorinated, deionized, and carbon filtered, but its use is not encouraged. Uncontaminated natural sediment is recommended.	Laboratory well water characterized as having a total hardness and total alkalinity as $CaCO_3$ of 34 to 46 and 17 to 19 mg/L, respectively, a pH range of 6.2 to 7.0, and a specific conductance range of 210 to 250 µmhos/cm. Monthly analysis of the water source indicated a TOC 0.55 mg/L for February 2009. Natural sediment (Batch No. 091808) collected from Glen Charlie Pond, Wareham, MA. The sediment was wet-pressed sieved (2.0-mm) prior to use.
Does water support test animals without observable signs of stress?	Yes.
Quality Of Water If problems are observed in culturing or testing of organisms, it is desirable to test water quality. Particulate, TOC, COD should be <5 mg/L and residual chlorine <11 µg/L	There were no apparent problems with water quality. During the study, ammonia levels (as N) in the overlying water were ≤2.4 mg/L.
Water Temperature 23°C for both species. The mean and instantaneous temperatures should not vary from the desired temperature by more than 1°C and 3°C, respectively.	Daily: 22 to 24°C Continuous: 23 to 24°C
pH Should not vary more than 50%. Survival is best at pH >6.5 for *C. tentans.*.	6.4 to 7.1
Dissolved Oxygen Maintained between 40 and 100%.	5.9 to 8.2 mg/L (≥69% ASV at 23°C; reviewer-calculated)

Guideline Criteria	Reported Information
Total Hardness Should not vary more than 50%. *H. azteca* are sensitive to hardness (e.g., they are not found in waters with calcium at <7 mg/L and DO at <2 mg/L).	40 to 48 mg/L as $CaCO_3$
Conductivity Should not vary more than 50%.	280 to 300 µmhos/cm
Sediment Characterization All sediment must be characterized for: pH, ammonia concentration of pore water, organic carbon content (total organic carbon (TOC)), particle size distribution, and percent water content.	Particle distribution – 83% sand, 12% silt, 5% clay (loamy sand; reviewer-derived from USDA soil texture triangle) Organic carbon content – 5.1% Solids – 49.71% pH – 6.0 Ammonia concentration of pore water – not reported
Additional Sediment Analysis BOD, COD, cation exchange capacity, Eh, pE, total inorganic carbon, total volatile solids, acid volatile sulfides, total ammonia, metals, synthetic organic compounds, oil and grease, petroleum hydrocarbons, and interstitial water analysis.	None reported
Laboratory Spiked Sediment Material should be reagent grade unless prior evaluations dictate formulated materials, etc.; Must know the test material's identity, quantity of major ingredients and impurities, water solubility, estimated toxicity, precision and bias of analytical method, handling and disposal procedures.	<u>Cypermethrin Technical 40/60</u> Synonym: FMC 30980 IUPAC Name: (*RS*)-α-cyano-3-phenoxybenzyl (1*RS*,3*RS*;1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate CAS Name: cyano(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate CAS No.: 52315-07-8 Description: not reported Lot No.: PL07-0633 Purity: 40.6% cis-isomer, 59.4% trans-isomer Storage: dark, room temperature

Guideline Criteria	Reported Information
Stock Solutions Test material should be dissolved in a solvent prior to mixing into test sediment; If solvent is used, both solvent control and negative control are required.	Two hundred (200) mL of a 25-µg a.i./L primary stock solution was prepared in acetone. From this, 100 mL of a 2.5 µg a.i./mL secondary stock solution was prepared in acetone. Five individual dosing solutions were prepared using a combination of the primary and secondary stocks, and bringing the mixture to 10 mL with acetone. All stock and dosing solutions were clear and colorless, with no visible un-dissolved test substance. Negative and solvent controls were included in the test.

Guideline Criteria	Reported Information
Test Concentrations For Spiked Sediment For LC50 calculation, test concentrations should bracket the predicted LC50; sediment concentrations may be normalized to factors other than dry weight (e.g. organic content, acid volatile sulfides); Sediment may be mixed using rolling mill, feed mixer or hand mixer.	A jar-rolling technique was used to apply the test substance to the sediment. A 9-mL volume of the appropriate prepared dosing stock solution (in acetone) was applied to 0.050 kg of fine silica sand in glass Petri dishes, and the solvent was allowed to evaporate off for 45 minutes. The dry sand was then added to 3.0 kg of wet sediment (total of 1.54 kg dw) in individual 1-gallon jars. Each jar was then rolled for 4 hours at room temperature at approx. 15 rpm. The jars were stored upright at 4 ± 2°C during conditioning. The treated sediments were allowed to equilibrate for a 14-day period in the refrigerator. Twice a week during the conditioning period and prior to addition to the exposure vessels (day -1), the jars were mixed on the rolling mill for an additional 2 hours at room temperature to ensure the sediment was homogeneous. The range of concentrations (6.3 to 100 μg a.i./kg) was based upon the results of a preliminary range finding study.
Test Aquaria 1. Material: Glass or stainless steel or perfluorocarbon plastics. 2. Size: 300 ml high-form lipless beakers containing 100 ml of sediment and 175 ml of overlying water.	300-mL glass vessels containing 100 mL (approx. 4.0-cm layer) of sediment (equivalent to 43 g dw) and 175 mL of overlying water. The total overlying water plus sediment volume was maintained at *ca.* 275 mL. Test vessels were covered with 40-mesh Nitex® screen for drainage.
Type of Dilution System Daily renewal or a flow-through system may be used.	Intermittent flow-through

Guideline Criteria	Reported Information
Flow Rate 2 volume changes/day	2 volume additions/day
Aeration Dilution water should be vigorously aerated prior to use so that dissolved oxygen in the overlying water remains above 40% saturation.	None reported
Photoperiod 16 hours light, 8 hours dark at 500 to 1000 lux.	16 hours light, 8 hours dark; 500 to 640 lux
Solvents Use of a solvent should be avoided since they may influence the concentration in pore water. If used,it should not exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests. Acceptable solvents include triethylene glycol, methanol, ethanol, or acetone. Surfactants should not be used.	Acetone, 9 mL per 1.541 kg dw sediment. The acetone was allowed to completely evaporate during the mixing procedure.

D. Test Design

Guideline Criteria	Reported Information
Sediment Into Test Chambers One day prior (Day -1) to start of test: test sediment, reference sediment, and negative control sediment should be thoroughly homogenized and added to test chambers; Overlying water is added to chambers in a manner that minimizes suspension of sediment.	One day prior to the addition of amphipods (day -1), the test systems were established. Overlying water was gently added, and each vessel was placed under the renewal system.

Guideline Criteria	Reported Information
Renewal of Overlying Water: Renewal of the overlying water should be conducted on day -1 prior to the addition of organisms or food on day 0. For flow-through systems, the flow rates should not vary by more than 10% between any two chambers at any time. Proper operation should be verified by calibration prior to test initiation.	The overlying water was replaced twice daily using an intermittent delivery system in combination with a calibrated water-distribution system. The test system was calibrated before and after the test, and visually inspected at least twice daily for proper functioning.
Placing Organisms in Test Chambers: Should be handled as little as possible and introduced into overlying water below the air-water interface.	Amphipods were impartially assigned one or two at a time into intermediate test beakers until all beakers contained ten amphipods. The test was initiated when each intermediate beaker of amphipods was added to each respective test vessel.
Range Finding Test A definitive test will not be required if no toxicity is observed at concentrations of 100 mg/kg dry weight of sediment**.**	<u>Preliminary toxicity assessment</u> • Treated sediment equilibrated for 10 days • 10-day exposure at nominal levels of 0 (negative and solvent controls), 0.010, 0.10, 1.0, 10, and 100 μg a.i./kg • three replicates per level, each containing 10 organisms • Survival averaged 87 (negative control), 97 (solvent control), 87, 90, 87, 97, and 0%, respectively • Dry weight averaged 0.10 (negative control), 0.10 (solvent control), 0.09, 0.14, 0.08, and 0.08 mg, respectively
Monitoring the test All test chambers should be checked daily and observations made to assess organism behavior such as sediment avoidance.	Test vessels were observed daily for mortality and abnormal behavior.

Guideline Criteria	Reported Information
Nominal Concentrations of Definitive Test Control(s) and at least 5 test concentrations; dilution factor not greater than 50%. Concentrations above aqueous solubility may be used.	0 (negative and solvent controls), 6.3, 13, 25, 50, and 100 μg a.i./kg sediment
Number of Test Organisms 10 organisms per test chamber are recommended. 8 replicates per treatment should be used.	80 amphipods per level, with 10 amphipods per replicate vessel and 8 biological replicates per level An additional 24 replicates were maintained for chemical analysis
Test organisms randomly or impartially assigned to test vessels?	Yes
Feeding *C. tentans* in each test chamber are fed 1.5 ml of a 4 g/L Tetrafin7 suspension daily. *H. azteca* may be fed with a mixture of yeast, Cerophyl, and trout chow (YCT) at a rate of 1.5 mL daily per test chamber. A drop in DO levels below 2.5 mg/L may indicate over-feeding and feeding should be suspended in all treatments until DO levels increase.	1.0 mL of yeast, cereal leaves, and flaked fish food suspension (YCT) once daily.

Guideline Criteria	Reported Information
Water Parameter Measurements Conductivity, hardness, pH, alkalinity, and ammonia should be measured in all treatments at the beginning and end of the test. DO should be measured daily. Temperature should be measured daily in one test chamber from each treatment. The mean and instantaneous temperatures should not vary from the desired temperature by more than 1 and 3°C, respectively.	<u>Overlying water:</u> For all levels, total hardness, alkalinity, specific conductance, and ammonia concentrations were measured in a composite sample on Days 0 and 10. DO, temperature, and pH were measured in each replicate vessel on Days 0 and 10 and in one alternating replicate from each level on Days 1 to 9. Temperature was also continuously monitored in an auxiliary vessel in the water bath. <u>Pore water:</u> Redox potential, pH, ammonia, and dissolved organic carbon (DOC) were measured in a composite sample on Days 0 and 10.
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used. Concentrations should be measured in bulk sediment, interstitial water, overlying water, and stock solution.	Surrogate samples vessels were collected on Days 0 and 10, and concentrations of cypermethrin were determined in pore water and sediment (see Reviewer's Comments section). The sediment/pore water matrices were isolated by centrifuging for 15 to 30 minutes at 1200 *g*. Aliquots of the dosing stock solutions were analyzed for cypermethrin. In addition, treated sediment from all levels were analyzed for cypermethrin prior to the allocation of the sediment into the replicate vessels (following equilibration).

11. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes. This study was conducted in accordance with GLP Standards as specified in 40 CFR 160 with the following exceptions: the routine water, sediment, and food contaminant screening analyses.
Control Criteria Was control mortality ≤20%? Were control *C. tentans* an average size of ≥0.6 g?	Mortality: Negative control – 0% Solvent control – 2%
Percent Recovery of Chemical:	Procedural recoveries (QC samples) conducted concurrently with sample analysis: Sediment: 82.7 to 108% of nominal Aqueous: 97.1 to 114% of nominal (with one outlier of 161%)
Data Endpoints - Survival - Dry weight (determined by pooling all living organisms from a replicate and drying at 60 to 90°C to a constant weight) - Body length (amphipod only)	- Survival - Dry weight
Raw data included?	Yes, sufficient

Effects Data

Toxicant Concentration				Survival		Dry Weight	
Nominal (µg a.i./kg)	Mean Measured[a]						
	Sediment (µg a.i./kg dw)	Pore Water (µg a.i./L)	Overlying Water (µg a.i./L)	Mean %	% Inhibition[c]	mg per larvae	% inhibition[c]
Control	<LOQ	<LOQ	Not assessed	100	N/A	0.09	N/A
S. Control	<LOQ	<LOQ	Not assessed	98	2	0.10	-11
6.3	4.6	0.072	Not assessed	93	7.0	0.08	11
13	8.5	0.14	Not assessed	88*	12	0.06*	33
25	25	0.41	Not assessed	91	9.0	0.05*	44
50	44	0.65	Not assessed	76*	24	0.02[b]	78
100	91	1.6	Not assessed	6*	92	0.01[b]	89

[a] LOQ were equivalent to 0.22 to 0.24 µg a.i./kg for sediment samples and 0.0013 to 0.031 µg a.i./L for pore water samples.
[b] Excluded from statistical analyses due to significant effect on survival.
* Statistically different ($p \leq 0.05$) compared to the negative control.
[c] These values have been corrected from those reported in Table 7 on p. 40 of the study report, which has the inhibitions incorrectly shifted up one for all levels.

Other Significant Results:

Biological: After 10 days, survival averaged 100 and 98% for the negative and solvent controls, respectively, and 93, 88, 91, 76, and 6% for the mean-measured 4.6, 8.5, 25, 44, and 91 µg a.i./kg sediment levels, respectively. Differences at the 8.5, 44, and 91 µg a.i./kg levels were statistically-reduced ($p \leq 0.05$) compared to the negative control. However, as there was no statistical difference observed upon survival at the 25 µg a.i./kg dw level, the difference at the 8.5 µg a.i./L level was considered incidental to treatment. The 10-day LC_{50} (with 95% C.I.) was reported by the study author to be 61 (59 to 63) µg a.i./kg sediment, and the NOAEC for survival was 25 µg a.i./kg.

After 10 days, dry weight averaged 0.09 and 0.10 mg per larvae at the negative and solvent control levels, respectively, and 0.08, 0.06, 0.05, 0.02, and 0.01 mg per larvae at the mean-measured 4.6, 8.5, 25, 44, and 91 µg a.i./kg sediment levels, respectively. Differences at the 8.5 and 25 µg a.i./kg sediment levels were statistically-reduced ($p \leq 0.05$) compared to the negative control (the 44 and 91 µg a.i./kg levels were not statistically compared due to the significant effect on survival at these levels). The 10-day EC_{50} (with 95% C.I.) was reported by the study author to be 29 (24 to 34) µg a.i./kg sediment, and the NOAEC for amphipod growth was 4.6 µg a.i./kg.

Analytical: Concentrations of cypermethrin were determined on Days 0 and 10 in sediment and pore water only (see Reviewer's Comments section). Concentrations remained relatively constant in sediment. On day 10, recoveries were within ±28% of Day-0 results. Mean-measured sediment concentrations were 4.6, 8.5, 25, 44, and 91 µg a.i./kg sediment, representing 72, 65, 100, 88, and 91% of the nominal treatment levels, respectively. Concentrations of cypermethrin declined 9 to 68% in pore water from Days 0 to 10 (% changes were reviewer-calculated).

Nominal Sediment Concn. (µg a.i./kg)	Sediment, µg a.i./kg		Pore Water, µg a.i./L		Overlying Water	
	Day 0	Day 10	Day 0	Day 10	Day 0	Day 10
Control	<0.22	<0.24	<0.0013	<0.020	Not assessed	Not assessed
S. Control	<0.22	<0.24	<0.0018	<0.031	Not assessed	Not assessed
6.3	4.4	4.7	0.085	0.058	Not assessed	Not assessed
13	7.4	9.5	0.20	0.074	Not assessed	Not assessed
25	28	22	0.63	0.20	Not assessed	Not assessed
50	45	42	0.68	0.62	Not assessed	Not assessed
100	96	85	2.3	0.95	Not assessed	Not assessed

B. Statistical Results

Statistical analyses were performed on amphipod survival and growth (dry weight). Analyses were performed using the response values for each replicate test vessel within a treatment level. Percent survival data were arcsine square-root transformed prior to analysis.

A t-Test was used to compare the performance of the negative control and solvent control data. Both endpoints were statistically similar, and therefore the treatment groups were compared to the negative control data to determine treatment-level effects.

Normality of the data was evaluated using the Chi-Square Test, and homogeneity of variance was evaluated using Bartlett's Test or Cochran's Test. Data met both assumptions and were thus analyzed using Dunnett's Test at the 95% level of certainty. NOAEC and LOAEC values were assigned based upon significance.

The linear interpolation method was used to calculate the LC/EC_{50} values with associated 95% confidence intervals.

Analyses were performed using TOXSTAT Version 3.5 statistical software and mean-measured

sediment concentrations.

Survival:
LC_{50}: 61 µg a.i./kg 95% C.I.: 59 to 63 µg a.i./kg
NOAEC: 25 µg a.i./kg
LOAEC: 44 µg a.i./kg

Growth:
EC_{50}: 30 µg a.i./kg 95% C.I.: 25 to 33 µg a.i./kg
NOAEC: 4.7 µg a.i./kg
LOAEC: 8.7 µg a.i./kg

12. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer statistically analyzed data for day 10 survival and dry weight. For both endpoints the negative and solvent control data were compared using a Student's t-test; for dry weight a significant increase ($p<0.05$; 11%) was detected in the solvent control weights, relative to the negative control weights. The data for dry weight were further tested using Shapiro-Wilk's test to confirm normality and using Levene's test to confirm homogeneity of variances. The 44 µg a.i./kg dry weight data were excluded from analysis, due to a significant effect on survival. Dry weight data satisfied the assumptions of ANOVA, so the NOAEC and LOAEC were determined using this test, followed by William's test (dose-dependent response); for some reason, the Williams' test run using Toxstat 3.5 did not suggest the same NOAEC as the William's test run using Nuthatch (as part of the EC_{50} output), and because the value obtained using Nuthatch was lower, it is the one reported. There was at least one group with zero variance for the survival data, so the NOAEC and LOAEC for this endpoint was determined using the non-parametric Steel's Many-One Rank test. These analyses were conducted using Toxstat 3.5 statistical software. The LC_{50} and EC_{50} values were determined using the moving average and Probit methods, respectively. For survival, the moving average method was selected over the Probit method because the Probit method could not provide a sound estimate (Goodness of fit probability =0). The moving average method was run using Toxanal 2009 and the Probit method was run using Nuthatch statistical software.

All of the above statistical analyses were performed in terms of the mean-measured sediment and estimated pore water treatment concentrations. Sediment endpoints are also calculated on an organic carbon-normalized basis, based on the following equation using an average TOC of 5.1%:

$$\text{mg/kg OC} = \frac{\text{mg/kg dry weight}}{\text{kg TOC/kg dry weight}}$$

Based upon mean-measured sediment concentrations:

Survival:
LC_{50}: 54 µg a.i./kg — 95% C.I.: 49 to 59 µg a.i./kg
Slope: N/A
NOAEC: 25 µg a.i./kg
LOAEC: 44 µg a.i./kg

Growth (dry weight):
EC_{50}: 31 µg a.i./kg — 95% C.I.: 26 to 37 µg a.i./kg
Slope: 4.99±1.25
NOAEC: 4.7 µg a.i./kg
LOAEC: 8.7 µg a.i./kg

Based upon ESTIMATED[2] pore water concentrations:

Survival:
LC_{50}: 0.007 µg a.i./L — 95% C.I.: 0.007 to 0.008 µg a.i./L
Slope: N/A
NOAEC: 0.003 µg a.i./L
LOAEC: 0.006 µg a.i./L

Growth (dry weight):
IC_{50}: 0.004 µg a.i./L — 95% C.I.: 0.004 to 0.005 µg a.i./L
Slope: 4.99±1.25
NOAEC: 0.0006 µg a.i./L
LOAEC: 0.001 µg a.i./L

Based upon OC-normalized mean-measured sediment concentrations:

Survival:
LC_{50}: 1060 µg a.i./kg TOC — 95% C.I.: 960 to 1160 µg a.i./kg TOC
Slope: N/A
NOAEC: 490 µg a.i./kg TOC
LOAEC: 860 µg a.i./kg TOC

Growth (dry weight):
EC_{50}: 608 µg a.i./kg TOC — 95% C.I.: 510 to 725 µg a.i./kg TOC
Slope: 4.99±1.25

2 Freely dissolved pore water endpoints (ug/L) estimated as:
Mean measured bulk sediment conc. (*ug/kg-dw*) / [Fraction TOC (*kg OC/kg-dw*) * K_{OC} (*L/kg-OC*)]

NOAEC: 92 µg a.i./kg TOC
LOAEC: 170 µg a.i./kg TOC

13. REVIEWER'S COMMENTS:

The reviewer's NOAEC and LOAEC conclusions for survival and dry weight agreed with those of the study author; however, the LC_{50} and EC_{50} estimates slightly differed due to the different methods used to obtain these values. The reviewer's results were obtained using EFED-approved statistical programs, so they are reported in the Conclusions section.

While the reviewer's analysis detected a significant ($p<0.05$; 11%) increase in dry weight of solvent control amphipods, compared to negative control amphipods, the reviewer does not believe that this is evidence of solvent interference. While not directionally the same, the magnitude of the effect is similar to that in the lowest treatment level, which was not statistically different from the negative control.

Results were provided in terms of mean-measured sediment (bulk and OC-normalized) and estimated pore water concentrations in the Conclusions section of the DER.

Overlying water was not analyzed due to the pyrethroids' strong affinity to sediment (i.e., high K_{oc} values) and regular renewal of the overlying water. It was also reported that previous studies performed at the laboratory indicated that only negligible amounts of pyrethroids partition to overlying water (Springborn Smithers Laboratories Study Nos. 13656.6106, 13656.6107, 13656.6110, 13656.6111, and 13656.6112, Putt, 2005).

This reviewer notes that the concentration of cypermethrin measured in pore water likely reflects both "freely dissolved" chemical (i.e., chemical that is not sorbed onto particulate organic carbon (POC) or dissolved organic carbon (DOC) in addition to dissolved chemical that is sorbed to DOC. This finding is indicated by the fact that the extraction and analytical methods used in this study do not distinguish among the two phases of chemical (freely dissolved and DOC-sorbed). It is also indicated by the much higher measured concentrations of cypermethrin in pore water than would be expected based on estimated values using sediment cypermethrin concentrations, its Koc, and sediment total organic carbon (TOC). For highly hydrophobic chemicals like cypermethrin, DOC in pore water can substantially reduce its bioavailability and toxicity. It is further noted that the pore water estimated environmental concentrations (EECs) generated using the Agency's PRZM/EXAMS model are based on freely dissolved chemical. Therefore, some downward adjustment of these pore water toxicity values using appropriate methods (e.g., Koc and DOC concentration in pore water) will likely be needed when comparing these values to freely dissolved EECs generated using PRZM/EXAMS. Since the measured pore water concentrations of cypermethrin do not accurately describe the exposure to parent compound, endpoints from this study will not be expressed in terms of measured pore water concentrations. Instead, this reviewer has estimated freely dissolved pore water endpoints based on measured

concentrations in bulk sediment, the fraction of total organic carbon in bulk sediment (5.1%) and the mean K_{OC} (141,700 L/kg-OC, MRID 42129002) for cypermethrin. These estimated pore water endpoints, which are based on the freely dissolved test material (i.e., chemical that is not sorbed onto particulate organic carbon [POC] or dissolved organic carbon [DOC]), are consistent with the expression of aquatic estimated environmental concentrations (EECs) from PRZM/EXAMS. It is noted, however, that K_{OC} values for cypermethrin vary considerably depending on soil type (20,800 – 328,500 L/kg). This range of K_{OC} likely reflects differences in organic carbon composition and other soil properties used to determine K_{OC}. Therefore, these estimated pore water endpoints are subject to the same uncertainty in determination and application of K_{OC} for cypermethrin.

Nominal Sediment (µg a.i./kg)	Mean-measured Sediment (µg a.i./kg)	Estimated Pore Water (µg a.i./L)	OC-Normalized Sediment (µg a.i./g OC)
6.3	4.7	0.0006	92
13	8.7	0.001	170
25	25	0.003	490
50	44	0.006	860
100	91	0.01	1780

Analysis of the stock solution samples used to dose the test sediments ranged from 96 to 120% of nominal fortified concentrations. Pretest analysis of the spiked sediment following equilibration and prior to allocation into the replicate exposure vessels ranged from 84 to 120% of nominal concentrations.

In pore water (measured at each level on Days 0 and 10), the redox potential ranged from 210 to 310 mV, the pH ranged 5.2 to 5.7, the DOC ranged from 11 to 33 mg C/L, and the ammonia (as N) ranged from 3.5 to 17 mg/L.

The analytical method used to quantify cypermethrin in (natural) sediment was validated in December 2008. Fortified samples were extracted two to three times with methanol:purified reagent water and hexane; the extracts were combined and purified for analysis using solid phase extraction (SPE). Aliquots were analyzed using gas chromatography equipped with mass selective detection in negative chemical ionization mode (GC-MS/NCI). In samples fortified at 0.100 and 100 µg/kg, recoveries averaged 99.3 ± 27.7% and 77.4 ± 4.34%, respectively, with a limit of quantitation (LOQ) of 0.0252 µg a.i./kg.

The analytical method used to quantify cypermethrin in freshwater was validated in January 2009. Fortified samples were acidified and extracted twice with ethyl acetate; the combined extracts were reduced in volume using rotary evaporation (30°C) and taken to dryness under nitrogen (room temperature). The residues were re-constituted in 0.1% peanut oil in acetone and analyzed using gas chromatography equipped with mass selective detection in negative chemical ionization mode (GC-MS/NCI). In samples fortified at 0.00100 (sample LOQ), 0.00300, 0.0200, and 0.0500

µg/L, recoveries averaged 114 ± 3.82%. Due to the low concentrations being tested, the LOQ was set at 0.00100 µg/L; sample LOQ recoveries averaged 110 ± 16.1%.

It was reported that representative samples of the overlying water source were periodically analyzed for pesticides, PCBs, and toxic metals, and that none of these compounds were detected in any of the water samples analyzed in agreement with ASTM guidelines.

Definitive test dates were February 10 to 20, 2009.

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15. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Title: Day 10 % survival
File: 6601s Transform: NO TRANSFORMATION

t-Test of Solvent and Blank Controls Ho: GRP1 Mean = GRP2 Mean

GRP1 (Solvent cntl) Mean =	100.0000	Calculated t value =	1.0000
GRP2 (Blank cntl) Mean =	97.5000	Degrees of freedom =	14
Difference in means =	2.5000		

2-sided t value (0.05,14) = 2.1448 No significant difference at alpha=0.05
2-sided t value (0.01,14) = 2.9768 No significant difference at alpha=0.01

WARNING: This procedure assumes normality and equal variances!

Title: Day 10 % survival
File: 6601s Transform: NO TRANSFORMATION

Steel's Many-One Rank Test - Ho: Control<Treatment

GROUP	IDENTIFICATION	MEAN IN ORIGINAL UNITS	RANK SUM	CRIT. VALUE	DF	SIG 0.05
1	Neg Control	100.0000				
2	4.6	92.5000	52.00	46.00	8.00	
3	8.5	87.5000	52.00	46.00	8.00	
4	25	91.2500	48.00	46.00	8.00	
5	44	76.2500	36.00	46.00	8.00	*
6	91	6.2500	36.00	46.00	8.00	*

Critical values are 1 tailed (k = 5)

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
91	80	75	93.75	0
44	80	19	23.75	0
25	80	7	8.75	0
8.5	80	10	12.5	0
4.6	80	6	7.500001	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 56.45638

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	2.393742E-02	53.84591	49.34374	59.22644

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	2.383154	22.21424	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 1.916629
95 PERCENT CONFIDENCE LIMITS =-1.042161 AND 4.875419

INTERCEPT=-3.271098

LC50 = 50.89709
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC25 = 22.63461
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 10.91488
95 PERCENT CONFIDENCE LIMITS = 0 AND 48.51464

LC05 = 7.054522
95 PERCENT CONFIDENCE LIMITS = 0 AND 28.20666

Title: Day 10 Dry Weight
File: 6601w Transform: NO TRANSFORMATION

t-Test of Solvent and Blank Controls Ho: GRP1 Mean = GRP2 Mean

GRP1 (Solvent cntl) Mean =	0.0863	Calculated t value =	-2.3607
GRP2 (Blank cntl) Mean =	0.1075	Degrees of freedom =	14
Difference in means =	-0.0212		

2-sided t value (0.05,14) = 2.1448** Significant difference at alpha=0.05
2-sided t value (0.01,14) = 2.9768 No significant difference at alpha=0.01

WARNING: This procedure assumes normality and equal variances!

Title: Day 10 Dry Weight
File: 6601wr Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 0.0109
W = 0.9790

Critical W = 0.9040 (alpha = 0.01 , N = 32)
W = 0.9300 (alpha = 0.05 , N = 32)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Day 10 Dry Weight
File: 6601wr Transform: NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

SOURCE	DF	SS	MS	F
Between	3	0.0002	0.0001	0.4930
Within (Error)	28	0.0044	0.0002	
Total	31	0.0047		

(p-value = 0.6900)

Critical F = 4.5681 (alpha = 0.01, df = 3,28)
= 2.9467 (alpha = 0.05, df = 3,28)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.01)

Title: Day 10 Dry Weight
File: 6601wr Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	3	0.0066	0.0022	5.6659

Within (Error)	28	0.0109	0.0004
Total	31	0.0175	

(p-value = 0.0037)

Critical F = 4.5681 (alpha = 0.01, df = 3,28)
= 2.9467 (alpha = 0.05, df = 3,28)

Since F > Critical F REJECT Ho: All equal (alpha = 0.05)

Title: Day 10 Dry Weight
File: 6601wr Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG 0.05
1	Neg Control	0.0863	0.0863		
2	4.6	0.0825	0.0825	0.3803	
3	8.5	0.0600	0.0600	2.6624	*
4	25	0.0525	0.0525	3.4231	*

Dunnett critical value = 2.1700 (1 Tailed, alpha = 0.05, df [used] = 3,24)
(Actual df = 3,28)

Title: Day 10 Dry Weight
File: 6601wr Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Neg Control	8			
2	4.6	8	0.0214	24.8	0.0038
3	8.5	8	0.0214	24.8	0.0262
4	25	8	0.0214	24.8	0.0338

Title: Day 10 Dry Weight
File: 6601wr Transform: NO TRANSFORMATION

William's Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg Control	8	0.0863	0.0863	0.0863
2	4.6	8	0.0825	0.0825	0.0825
3	8.5	8	0.0600	0.0600	0.0600
4	25	8	0.0525	0.0525	0.0525

Title: Day 10 Dry Weight
File: 6601wr Transform: NO TRANSFORMATION

William's Test - TABLE 2 OF 2 Ho: Control<Treatment

IDENTIFICATION	COMPARED MEANS	CALC. WILLIAMS	SIG 0.05	TABLE WILLIAMS	DEGREES OF FREEDOM USED
Neg Control	0.0863				
4.6	0.0825	0.3803		1.7000	k= 1, v=28
8.5	0.0600	2.6624	*	1.7800	k= 2, v=28
25	0.0525	3.4231	*	1.8100	k= 3, v=28

s = 0.0197

6601W : Day 10 Dry Weight

Williams Test

[One-Sided Test for Decrease, alpha = 0.050000]

Dose	Isotone Means	T-bar	P-value	Significance
0	0.0863	.		
4.6	0.0825	0.4053	N.S.	
8.5	0.06	2.837	<0.005	*
25	0.0525	3.648	<0.005	*
44	0.0166	7.525	<0.005	*

"*"=Significant; "N.S."=Not Significant.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	14.	8.7	24.	0.11	0.60
EC10	17.	11.	26.	0.092	0.65
EC25	23.	17.	30.	0.064	0.74

EC50 31. 26. 37. 0.038 0.84

Slope = 4.99 Std.Err. = 1.25

!!!Poor fit: p = 0.045 based on DF= 2.0 35.

6601W : Day 10 Dry Weight

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	8.00	0.0863	0.0765	0.00972	100.	0.00
4.60	8.00	0.0825	0.0765	0.00597	100.	0.00189
8.50	8.00	0.0600	0.0763	-0.0163	99.7	0.263
25.0	8.00	0.0525	0.0516	0.000857	67.5	32.5
44.0	8.00	0.0166	0.0169	-0.000229	22.0	78.0